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08/08/09
ssDNA was extracted from 500 µl of phagemid preparation (see below) by 2 phenol extractions followed by ethanol precipitation. DNA was quantified by spectrophotometry with 1.0 A₂₆₀ nm equal to 40 µg/ml for ssDNA or 50 µg/ml for dsDNA. For GFP detection, cells were detached using a trypsin-EDTA mix (GIBCO BRL) and analyzed on a FACScan (Becton Dickinson).--

In accordance with 37 CFR §1.121 a marked up version of the above-amended paragraph(s) illustrating the changes introduced by the forgoing amendment(s) are provided in Appendix A.

In the Claims:

Please cancel claims 18-50 without prejudice.

Please amend the claims by substituting the following claims for the corresponding previously pending claims of the same number(s):

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1. A method of selecting a polypeptide that is internalized into a target cell, said method comprising:

i) contacting one or more target cells with one or more members of a phage display library displaying one or more polypeptides;

ii) removing and eliminating members of said library that are bound to the exterior surface of said target cells with a strong wash; and

iii) identifying internalized members of said phage display library that are internalized into one or more of said target cells, where said internalized members of said phage display library each display a polypeptide that is internalized into a target cell.

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4. The method of claim 1, wherein said identifying comprises recovering internalized phage and repeating steps (i) through (iii) to further select for internalizing binding moieties.

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11. The method of claim 1, wherein step (ii) is performed at about 4°C.

Please add new claims 51-56 as follows:

--51. The method of claim 1, wherein said method further comprises contacting the target cells with cells of a subtractive cell line.

52. The method of claim 51, wherein said method further comprises contacting the target cells with live cells of a subtractive cell line.

53. The method of claim 1, wherein said removing comprises contacting the target cells with a low pH wash.

54. The method of claim 51, wherein said removing comprises contacting the target cells with a low pH wash.

55. The method of claim 1, wherein said removing comprises contacting the target cells with a trypsin.

56. The method of claim 51, wherein said removing comprises contacting the target cells with a trypsin.

57. The method of claim 51, wherein the target cells are cells that are transformed a nucleic acid that encodes and expresses a target receptor and the subtractive cell line is the non-transformed cell line.--

These amendments are made without prejudice and are not to be construed as abandonment of the previously claimed subject matter or agreement with the Examiner's position. In accordance with the requirements of 37 C.F.R. § 1.121, a marked up version showing the changes to the claims, is attached herewith as Appendix A. For the Examiner's convenience, a complete claim set of the currently pending claims is also submitted herewith as Appendix B.

REMARKS

Status of the Claims.

Claims 1-17, and 51-57, are pending with entry of this amendment, claims 18-50 being cancelled and claims 51-57 being added herein. Claims 1, 4, and 11 are amended herein. The new